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THE LIFE HISTORY, THE NORMAL FISSION AND THE REPRODUCTIVE ORGANS OF PLANARIA MACULATA.

A Dissertation Presented to the Board of University Studies of the Johns Hopkins University for the Degree of Doctor of Philosophy,

by

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I. Introduction.

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The work of this paper has been carried on at the Johns Hopkins University during the three years preceding the date of its publication, and I wish to express my sincere gratitude to Professor W. K. Brooks for his kindly help and guidance throughout the course of my study. I am also indebted to the authorities at the Marine Biological Laboratory, Wood's Hole, Massachusetts, for the privileges extended to me by that institution in the course of my work there during the past three summers.

The absence of reproductive organs during the entire year in Planaria maculata from certain localities and the appearance of these organs regularly each year in others aroused my interest and led me to seek some explanation. While accumulating data upon the presence or absence of these organs in different localities and seasons I found in the localities where reproductive organs are not developed abundant material for a study of the normal fission, and since the normal fission of Planarians while mentioned by many writers during the past ten years has not been ade-

quately described or figured in a single instance, with the possible exception of Planaria fissipara (Kennel '88) which presents just the opposite of the type of fission in Planaria maculata, I have attempted a thorough description of this process as it occurs in Planaria maculata. The entire absence of reproductive organs in many localities remains considerable of a mystery, but I give the data thus far obtained under the head of life history. Finding that the reproductive organs of this common form had never been described I gave last year a short note on their anatomy and development and these observations are further elaborated in this paper.

II. THE LIFE HISTORY OF PLANARIA MACULATA IN DIFFERENT LOCALITIES.

With a single exception all the Triclads which I have found described as reproducing a sexually are without reproductive organs during the period of fission. The one exception is the Land Planarian Placocephalus Semperi mentioned by Von Graff (99, p.224). There is also to my knowledge but a single exception to this in the Rhabdocoeles,

viz., Microstomum (Sekera, '88). Since all Planarians remain for a long time after hatching without these organs it might easily be supposed that it was during this post-embryonic period that the asexual reproduction occurred. In a hypothetical case we might suppose the young hatched in the spring and early summer and from then until the fall more or less asexual reproduction. In the fall this ceases and the reproductive organs make their appearance. After the egg-laying in the following spring the adults might die or they might lose their reproductive organs and live on. In the latter case they would at the end of the summer be indistinguishable from the young of that year. If it could be proved that the adults all die after their egg laying, i.e., lived but one year, we should then have a kind of alternation of generations in which all the individuals resulting from the sexual multiplication became sexually mature. It it could not be proved that all the old ones died off we should have the possibility and even the probability that their reproductive organs disappeared and they again passed through a season of asexual reproduction with the younger worms. We should then represent the life history as made up of alternate periods of asexual absence of reproductive organs in all the worms of a local-

ity at certain times and the abundant occurrence of normal fission.

In one locality where I have observed them for three years Planaria maculata goes through some such series of changes as that I have just indicated with the probability that the old ones live on after the egg-laying. The point of interest is that there seem to be different courses in different localities. I have taken systematic observations in a number of places during the past three years, the results of which can best be presented by a statement of the data obtained. I should preface this by saying that I have taken as many precautions as seem to me necessary to guard against the possibility of my dealing with two distinct species or two varieties of a species where but one has been previously recognized. There are no external differences between the worms of the several localities beyond the variations in color which one might expect. Specimens from a single locality will often differ more in color than any specimens collected from a locality where the life history is entirely different. So far as I can ascertain from the most careful measurements the shape of the head and other proportions are identical in all the localities.

What I have learned regarding the changes in the

several localities is as follows:

Locality (1). All the worms are large and sexually mature in the spring. During May and June they lay their eggs. In July the young and adults are found together and are easily distinguishable. In the latter part of July and during August the adults are found with their reproductive organs in all stages of degeneration and sometimes show evidence of fission before these have entirely disappeared. Toward the end of August and in early September no reproductive organs are to be found in any of the individuals and the old ones if still alive cannot be distinguished from the young. They are found during these two months reproducing by fission to a very considerable extent. In the fall the fission stops and all the individuals develop reproductive organs in preparation for the egg-laying of the next spring. This has been observed for three years.

Locality (2). The same as locality (1), save that I have never seen any satisfactory indications of fission. This is then, as far as the observations go, an exclusively sexual locality. Observed the same course for three years.

Locality (3). A period of very active fission was observed during June, July, and August. No reproductive organs have ever been discovered, but a single egg-capsule

was once found which shows that sexual individuals do sometimes occur. Observed for one year.

Locality (4). A period of very active fission as in locality (3) which begins in June and lasts until the last of September, but no reproductive organs or traces of the egg-capsules have ever been seen. Specimens examined in November and again in May showed no sign of reproductive organs. Observed the same for two years. The account of the fission in Planaria maculata given by Randolph ('97) was based upon worms from this locality.

In another locality which I have kept track of for one year there seems to be the same condition as in locality (1).

These observations are based upon many more individuals than those I have been able to stain whole, or to section. The egg-capsules remain upon the stones for three or four months after the young worms have broken from them and afford unmistakable evidence of the occurrence of a reproductive season even though no sexual worms are found. In like manner when the capsules have never been found, though the under sides of thousands of stones were examined in collecting and I was constantly looking for them, it amounts to a disproof of any extensive egg-laying in a giv-

en season which alone would be sufficient without the additional evidence obtained from the absence of reproductive organs in all the specimens examined. When worms have their reproductive organs even partially developed the genital pore can be readily seen on the ventral side and as they approach sexual maturity the two vasa deferentia full of sperm are very noticeable on the ventral side as two white cords on either side of the pharynx. This makes it possible at the egg-laying season to determine the presence or absence of reproductive organs in every specimen collected. These facts are familiar to every one who has studied Planarians, but I mention them in order to show the number of specimens examined to furnish the basis for the statements regarding the occurrence or absence of the reproductive organs in the respective localities.

As to the interpretation to be put upon these conflicting data; if I had only localities (2) and (4), the one with no evidence of fission, the other with no sign of sexual organs, I should conclude that there were two varieties of Planaria maculata, one of which reproduced almost entirely by fission and in which reproductive organs were very rarely found. Locality (1), however, shows that fission may occur in what I have termed a sexual locality, and

locality (3) shows that an asexual locality may sometimes produce worms with sexual organs. The gradation which the four localities present can, it seems to me, be explained by supposing that Planaria maculata having the power of fission which is common to many Planarians this method of reproduction has been in some localities substituted almost entirely for the sexual method. Whether this substitution is permament or only temporary can, of course, only be ascertained by observations extending over a number of years.

The fact that all the specimens collected from a sexual locality are found each fall with reproductive organs developing shows that this species becomes sexually mature the year after hatching, whereas Ijima ('84) found that the young of Dendrocoelum lacteum did not develop any reproductive organs until the second year. Planaria simplissima, which I followed through the fall and winter at Williamstown, Mass. (1900), showed exclusively sexual individuals during that time. The ovaries and testes were at first found in their younger stages, but in the early spring they were so far developed as to indicate the nearness of the sexual season. It is therefore probable that this form also develops its reproductive organs the first year.

III. THE ACT OF NORMAL FISSION AND THE EXTERNAL CHANGES FOLLOWING.

Jacob Keller ('94) in an article based upon the in-investigation of normal fission in Rhabdocoele Turbellaria (Stenostoma and Microstomum) and the regeneration of fresh water Planarians, has tabulated the various modes of normal fission in Triclads and Rhabdocoeles. This and his review of the literature make any such attempt on my part unnecessary. I will merely indicate the schema of the types of fission according to Keller and the place in this which the type found in Planaria maculata occupies. He divides the normal fission of Triclads into two types (1) that in which the new organs develop previous to the separation of the new formed individuals (Planaria fissipara, Kennel, '88,) and (2) that in which the development does not begin until after the new individuals have separated (various Land Planarians and Planaria albissima, Sekera, '88). Such a distinction is not hard and fast, because the process in Planaria subtentacula, (Zacharias, '86) is some where between these two conditions. The first type is similar to the fission which occurs in many Rhabdocoeles, the second is in some cases a simple fragmentation and with difficulty

distinguished from the regeneration following accidental mutilation. Planaria maculata supplies just the type of fission necessary to make a very complete series from the one extreme to the other. Briefly, this series is as follows:

(1) In the normal fission of Planaria fissipara (Kennel, '88) a constrict appears a short distance behind the mouth and a new worm is produced at the posterior end of an old one. Before the two separate the new individual has begun to feed for itself and its eyes, brain, gut, etc. are in the same condition as those of any normal adult specimen. As the time for separation approaches a furrow makes its appearance at the place of division and deepens slowly as the internal changes progress. There is little difficulty in recognizing individuals about to divide, as for some hours before the separation we have two perfect worms with the head of the smaller attached to the tail of the larger by a narrow isthmus. This type is the same as is found in all Rhabdocoeles.

(2) In Planaria subtentacula (Zacharias, '86) the separation occurs when the new organs of the tail piece are partly formed. The posterior gut rami are fused in front of the new pharynx, but the formation of the head has been

delayed. There is an external furrow which is the first sign of the approaching fission and is visible until the separation occurs four days later. My figure of a Planaria maculata on the third day after normal fission (Figs. 5 and 17) shows the condition of a tail piece of Pl. subtentacula immediately after fission.

(3) The division in Pl. maculata is at a corresponding place behind the pharynx, but is preceded by no external furrow, internal development of organs or histological changes along the line of separation so far as I can discover (Fig . 22). The animal pinches itself in two at a definite place (Figs. 24 and 25) and the head and tail pieces after division are as though cut in two with a knife a short distance behind the pharynx. The occurrence of the division at a corresponding place links this with (1) and (2), while the separation without any previous rearrangement of old or development of new organs makes the fission of Pl. maculata almost the same as the last.

(4) This is found in many Land Planarians (Lehnert '91, v. Graf, '99, Bergendal, '87, Fletcher and Hamilton) when one or more pieces of varying lengths are pinched off at the posterior end and each fragment on regenerating the necessary organs becomes a normal individual.

Although Planaria albissima (Sekera, '88) in which the division occurs just anterior to the pharynx might be used to fill the place (3) which I have assigned to Planaria maculata, the latter completes the series in a more satisfactory manner since its division is posterior to the pharynx and in exactly the same place as (1) and (2).

On reviewing the accounts of the different authors (Zacharias '86, Kennel, '88, Sekera, '88, v. Graff, '99)and others, one finds nowhere given a complete description with adequate figures of either the external or internal changes from day to day in a normally divided Triclad. The external changes have been quite well described by Kennel ('88) in the single case of Planaria fissipara, which is a different type from Planaria maculata, and some of the papers reviewed by von Graff, ('99) seem to have indicated pretty well the simple external changes of the Land Planaria. The histological changes connected with normal fission have been quite generally neglected. Although Keller ('94) mentions them as similar to those following the regeneration after artificial mutilation, he gives no figures of the process and seems to have studied mainly the regeneration after artificial cutting.

The occurrence of fission in Pl. maculata was first

noted by Miss Randolph ('99) in a paper upon the regeneration of this form. This is, however, only a brief mention and there is no attempt at a detailed description. As so much study is being devoted to the regeneration after artificial mutilation of Planarians it seemed to me worth while to offer a thorough description of every phase I could observe of the normal fission and subsequent regeneration of Pl. maculata and this is embodied in the following:

If previous to the normal fission of Planarian maculata there is any external furrow to indicate the place of division it can last but a very short time for many of my specimens divided within a few minutes after I had scrutinized them very closely for some trace of a furrow and found none. This is illustrated by Fig. 22, a whole specimen which was found divided (Figs. 24 and 25) soon after it was drawn. I believe therefore that the single case where I saw the actual separation was the normal process. In this case the worm just after being transferred upon a spatula from one dish to another pinched itself in two apparently by a quick muscular contraction. There was nothing more to the process. (Figs. 22 and 24 - 25). The two pieces moved away without inconvenience and were exactly alike all newly divided pieces found in the ponds or the

laboratory (Figs. 2 and 19). During the collection at different times of over three thousand worms for my experiments I was constantly on the watch for specimens with some furrow or constriction at the place of division and I isolated many specimens which I fancied were thus marked. These invariably failed to show any furrow when they were fully expanded and could be more carefully observed. Some of them divided several days later, but the absence of any furrow had been previously determined by several close examinations.

The examination of a large number of whole worms at the season when the fission is at its height did not show any with signs of new organs developing as a forerunner of fission. Serial sections of tail pieces killed soon after the division also show no trace of the new pharynx, brain, or any change in the gut. The making of the new organs and rearrangement of the old is hardly apparent externally until the second or third day after the fission occurs, although within twenty-four hours the parenchyma cells at the anterior end cover the naked scar left by the fission with a delicate epithelium and form a small cap of new tissue which increases in size from day to day (Figs. 3, 12, 13).

These facts seem to show that the worm when it is ready for division quickly pinches itself in two at a definite place behind the pharynx and explain why out of one hundred and eighty-six divisions which occurred in the laboratory I saw the process in only one case though many individual specimens were examined at frequent intervals for days at a time.

As I can discover no histological changes at the place of fission in the parenchyma or other tissues of the whole worms which I have sectioned, I believe that the division is accomplished by a constriction of the circular muscles which can only take place when the animal is of a suitable size and proportions for division, and in this latter respect differs from what is found in some of the Land Planarians (v. Graff ('99), Bergendal ('87), which will almost always divide if sufficiently irritated.

Wilson (1900) has recently explained the fragmentation of a Nemertean (Cerebratulus lacteus) as due to a violent muscular contraction not preceded by any discoverable histological processes which can aid the separation. The Nemertean fragmentation may be quickly brought about by almost any stimulus which irritates the animal and may cut through opposite any one of the gut lobes. The effect of

irritation here and in the Land Planarians led me to try the effect of irritating Planaria maculata in various ways.

The killing fluids used never whole worms to separate as frequently happens with the Land Planarians. No amount of irritation with blunt instruments produced any result. Many small cuts at different places on the surface transverse cuts which almost divided the specimen at the place where normal division occurs or at any other points, and irregular mutilations at different places are all quickly healed and do not produce any more divided specimens in a lot of twenty or twenty-five worms than are to be found from day to day in lots which have not been thus mutilated, and it is the same when single specimens are isolated. According to my records the division was even less in lots so treated. It seems, therefore, that ordinary irritation will not produce the division, which must be considered a spontaneous and perfectly normal process serving the purpose of multiplication. The facts which were noted at the beginning of this paper upon the absence of reproductive organs in worms from localities where fission occurs extensively further demonstrate that the multiplication of the species in such localities must be accomplished almost entirely by this asexual method.

The only influences by which I succeeded in regulating the fission were food and a fresh supply of water. These were effective only in a general way. If twenty or twenty-five worms are isolated and not fed some of them will be found divided from time to time during the first three or four days. They may be kept in this way for many weeks, but after the first few days, even though the water is frequently changed the divisions are so rare as to be practically *nil* and might easily be explained by their obtaining food from eating one another, a thing which frequently happens. On the other hand, if they are kept well fed and in clean water such a lot of worms will have several divided specimens almost every day and these pieces will often redivide before they have reached normal proportions. The amount of division is therefore directly dependent upon the amount of food. It can, however, be checked by foul water.

In feeding the worms upon crushed snails the water was often fouled and could not be replaced as soon as would have been desirable by the pond water in which they were kept. In such cases the fission ceased, but six to ten hours after changing the water there seemed to be an epidemic of division. My records show that I obtained as many

divided specimens in the twelve hours following such changes as in all the other times of my experiments, although the ratio in time was only 1:10. This proportion can hardly be considered an accident, but I think the point is in need of more careful investigation.

All that these experiments indicate is that the worms will not divide unless well fed, and that even when they are ready for division it may be prevented by an abnormal condition like the foulness of the water. This emphasizes the necessity of having all the conditions as favorable as possible in any such experiments.

In the laboratory the fission occurred between 10.00 P.M. and 6.00 A.M. in thirty-nine specimens out of forty-two in which the approximate time of division was recorded. This did not seem due to the amount of light which the animals were subjected to during the day, for some of the dishes were so shaded that there was practically no light day or night except when they were being examined, and the division was the same in these as in others which were exposed to the full daylight. Lehnert ('91) observed in Bipalium kewense that the division took place at night.

In trying to show that the fission occurring in the laboratory is a normal reproductive process I have taken

pains to show that it occurs to an even greater extent in nature. To do this I collected some three thousand worms from different localities and found them with the marks of more or less recent division always abundant. The following count of the specimens collected at one time is representative. Whole, 75, heads, 110, tails, 60, odd mutilations, evidently the result of collecting, 5. These figures are good in asexual localities at any time from June to October and when it is known that tail pieces may redivide after five days and heads after about twice that time one realizes how much multiplication may be thus effected in a short time. A smaller mumber of tails is constant in about the above proportions. The tails are also in the minority in the laboratory unless isolated, from the fact that they are eaten by the whole worms and their own head ends and even when isolated they sometimes die apparently from becoming entangled in their own mucous secretions. As they are without the pharynx or sense organs of the head. (Figs. 3,12, - 13, and 28), there is probably more destruction of heads than of tails in nature where they may be preyed upon by other hunters than their own kind. Another partial explanation of the smaller number of tail pieces in my count is that they become almost indistinguishable from whole

worms after the sixth or seventh day, while in head pieces the proximity of the pharynx to the posterior end remains for ten or twelve days as a sure evidence of recent division (Figs. 29 and 30). The abundance of tail pieces in all stages of regeneration is sufficient proof that a large proportion of them do become perfect worms. There is no limit of growth which when reached causes the fission since the heads and tails when collected are of all sizes. (compare Fig. 19, 20, and 24 with Figs. 2, 13, 16).

The figures on plates I. and II. which illustrate the following description of the external changes were all made from living specimens which were kept well-fed upon a darkly pigmented pond snail. The presence of this food in the digestive track enabled me to follow the changes of the gut by measurements of the same specimen on successive days In the figures I have only represented the main gut branches as the smaller ones could not be accurately made out in the live worm and if shown would only obscure the course of the larger ones.

After their normal separation the heads and tails follow the same course as though artificially cut in two (Figs. 2-7, 16-18, 19, 28, 29, and 30). I have no observations upon any differences between the processes follow-

ing normal and those following artificial separation. Viewed from the dorsal or ventral side the naked end of either piece immediately after division shows a straight transverse edge with just a little line of white parenchyma protruding beyond it (Figs. 2, 24 and 25). Viewed head on the scar is like a transverse section of the worm somewhat reduced in size. The sides of the body are curved in slightly as though the scar were always kept a little drawn together. If the pieces contract as they frequently do a crescentic area of scar tissue appears and just behind it a very dark line produced by the drawing together of the pigment flecks (Figs. 1, 21, 24, 26). Sections show the parenchyma to be naked and occasionally portions of the gut are in contact with the outside (Fig. 35).

During the first twenty-four hours the cut end becomes pointed by the appearance of a small triangular area of white scar tissue and in the tail piece (Figs. 3 - 7 13-14, 16-18) this forms the part of the new head lying anterior to the tips of the auricles. In the head piece it merely caps the posterior end (Figs. 19, 28-29). During the second day this new tissue increases in size. On the third day the first indication of the eyes is found as two minute dots just on the line between the old and new

tissues (Figs. 4, 14 and 16). The halo about them is first seen when the pigment flecks become apparent on the fourth or fifth day (Figs. 6 and 18). The area of the new tissue has by the fourth day become considerably larger and its anterior edges have begun to make the angles of the normal head (Figs. 5 and 17). The points of the auricles do not show plainly until the sixth or seventh day (Figs. 7 and 8) and even on the tenth day although the head has reached its normal shape it may be much lighter in color and so indicate the recent fission. From the sixth day on it is difficult to distinguish a tail piece from a normal specimen and many tail pieces even of this age are probably counted as whole specimens in collecting. (Figs. 7 and 8)

A word explaining some of the figures will review these changes. Figures 2-7 represent the changes of a single tail piece on six successive days and figures 20 and 21 represent this piece redivided on the eighth day. Figures 16-18 represent a single tail piece on the third, fourth and fifth days after normal fission and figures 13 and 14 another tail piece on the second and fourth days. Figure 8 is a tail piece the eighth day after separation. All the figures of the external changes were made from live specimens studied under a dissecting microscope. They are

as accurate as the constant movement of the extended animal will allow and were drawn only after careful measurements of the length, breadth and other proportions with a pair of compasses. For the sake of clearness the finer branches of the gut are omitted.

When the tail is pinched off there has been no rearrangement of the gut and we therefore have two parallel gut rami with their various branches (Figs. 2, 21 and 25). The gut in whole worms of this species has, however, so many branches and secondary connections that it is exceptional to find but two long rami in the posterior end (Figs. 22, 23, 29 and 31). Three or four is the common number and there are sometimes as many as five. Where there are but two main branches the changes are easily followed. As late as the third day the anterior ends may remain separated (Fig .4), but usually on the third day they have met and fused together (Fig. 16). During the third and fourth days this fusion has extended further back (Fig. 17). A small pharynx, not as yet connected with the gut, is visible on the fourth day in the live animal (Figs. 5 and 17). The fused rami make themselves over anteriorly into the single anterior gut ramus of the Triclad with no apparent increase in the amount of tissue involved (Figs. 17-18 and 5-6). The

new pharynx is connected with this posteriorly by the end of the fourth day. On the fifth day (Figs. 6 and 14) the pharynx may be protruded and used in feeding though as yet quite small. In the tail pieces the pharynx develops somewhat anterior to its final position (Figs. 5 and 9). From the fifth day (Fig. 6) on, the only change is the slow rearrangement of the proportions as the pharynx moves back to its normal position or if the pharynx be taken as the fixed point the old tissue may be said to move around in front of it. This remodeling of the contour is similar to that described in all studies of regeneration (Morgan, 1900 and '98, and Bardeen, 1901).

As will be seen from the figures (2, 12, 13, 21, and 25) the portions of the gut found in tail pieces are hardly the same in any two cases, but the same formation of a single ramus always follows when the several rami unite at their anterior ends (Figs. 6, 14 and 18). The fission is probably the cause of the complexity in the branching and interconnections in all parts of the gut in Planaria maculata (Figs. 14, 20, 22, 23, and 31). Many other Planaria have long branches extending from well up on the posterior rami toward the posterior end, while anteriorly the gut is quite regular. If fission occured to any extent in these

forms it would produce the same kind of irregularity as is found in Planaria maculata. Indeed, the production of these bizarre connections can often be actually followed as in Figs. 13 and 14). Zacharias, ('86) was struck by the great complexity of the gut branching in Planaria subtentacula, but did not correlate it with the fission in that species.

In many cases the tail pieces redivided before tha pharynx had reached its normal position or the proportions of the adult had been attained (Figs. 20-21). The only requisite for a new division seems to be plenty of food and time to accumulate enough material behind the pharynx to make a new worm out of. Because of the latter condition tail pieces redivided much sooner than heads. The shortest time in which a head redivided (tenth day) was just twice that in which tails frequently redivided.

In the laboratory the intervals between divisions were so irregular that nothing can be said except that tail pieces redivide much sooner than the corresponding heads. One must suppose that in nature some individuals go for a longer time than others without division, since the heads and tails when collected are from worms of all sizes.

To show the irregularity between the acts of normal

fission I have inserted a text figure which has for its basis the record of three individuals which were isolated and followed for 31, 33 and 32 days respectively. Beginning with the whole specimens 1, 2, and 3 on the left, their acts of normal fission into a head and tail piece are indicated by the divergence of the horizontal lines and the time at which each occurred by the scale of days given below. I have similar records of quite a number of the products of division for a longer or shorter time than the above, but these three cases sufficiently indicate the entire lack of any observed regularity in the intervals between one act of fission and the next.

In the head piece after the normal fission the remodelling goes on in such a way that the portion anterior to the pharynx remains the same size, or at least the most careful measurements do not show any change in its proportions, while the portion posterior to the pharynx grows until it has completed the normal proportions. Figures 19-28 and 29-30 will serve to illustrate this and also Fig. 11 which represents the changes in outline at the posterior end of a head piece during the seven consecutive days following normal fission. Apparently the rapid growth of the animal when fed masks to a certain extent the rearrange-

ment of proportions, morpholaxis, which is so evident in pieces artificially cut from different parts of the worm for experiments in regeneration (Morgan, '98 and 1900) and in the tail pieces (Figs. 2-7) which must go without food until the new pharynx develops.

IV. INTERNAL CHANGES AFTER FISSION.

Before describing the histological processes involved in the regeneration after normal fission it is necessary to describe the parenchyma of this species and I also take occasion to review to some extent the various descriptions which have been given by different authors of the normal parenchyma and its part in regeneration.

Leaving out the gland cells, muscle and nerve fibres and other organs which are set in the parenchyma as a matrix the parenchyma (Figs. 42 and 43) in an adult _Pl. maculata_ has the appearance of a syncitial mass the cytoplasmic portion of which is relatively large. The structure of the cytoplasm is such that it has after fixation a varying number of vacuoles and irregular clefts surrounded by a substance made up of what resemble the fibres of a net-work. The nuclei (np) which seem properly to belong

to this syncitium are set irregularly through it and as in a typical syncitium have no trace of a cell-outline. Where the clefts and vacuolee are greatly increased in size and number the tissue has something the appearance of scattered multipolar cells, the processes of which are everywhere continuous with those from neighboring cells. This condition is what is more frequently observed in the central parts of the adult Planarian, but around the edges of the body and at the ends there is a closer resemblance to the condition of the parenchyma in an embryo for here the nuclei are more closely set and there is so little cytoplasm between them that large vacuoles or clefts are absent (Fig. 41).

In Pl. maculata the adult parenchyma has much more the appearance of a syncitium which has its cytoplasm very greatly vacuolated than of a large number of multipolar cells which are connected by their branching processes. The latter, however, is evidently the structure in some forms for Krsmanovic ('99) says (p.188), "Das Mesenchym ist auch bei meinen beiden Formen von reticulärem Bau, wie dies die Fig. 9 (me) deutlich zeigt. Die Ausläufer der zellen verbinden sich zum Theil unter einander, zum Theil umspinnen und umgreifen sie in Form von Fasern und Platten."

Ijima, ('84) also described the parenchyma of Planaria polychroa as made up of anastomosing cells.

Whether the parenchyma is a vacuolated syncitium or made up of connecting multipolar cells is of course of little importance for there may be differences in the parenchyma of different species of Planaria such as Böhmig ('91) describes in the Rhabdocoeles. I have also observed that fixation with plain corrosive sublimate makes the vacuoles more irregular and gives an appearance very much like Ijima's ('84) description, while such a fluid as Zenker gives the more regular appearance shown in my figures with evenly rounded vacuoles.

In the foregoing description I have mentioned as belonging to the parenchyma the cells of a syncitial mass. In addition to these there are scattered through the syncitium other cells of a distinctly different type which have a large nucleus and a neucleolus which is surrounded by a clear space (Figs. 42 and 43 s). The outline of the cell cytoplasm is distinct and if not sharply spindle-shaped usually extends out much more from either end than from the sides of the oval nucleus. While these individual cells are of a distinctly different type from the fused cells of the syncitium, there are so many others (Figs. 42 and

43) intermediate between the two it seems a fair presumption that there is a transition from one to the other, but it is not so clear in which order the series can be interpreted.

There is nothing particularly new in the above description, but I give it in detail and also the review which follows in order that my description of the regenerative processes may be as complete as possible.

There is substantial agreement in the observations of Ijima ('84), Hallez ('79), Chichkoff ('92), Woodworth ('91), and Krsmanovic ('98) upon the parenchyma of the Planaria. Böhmig ('91) gives the following resumé of his conclusion regarding the parenchyma of the Turbellaria as a whole. He says, (p. 205), "Das Parenchym der Turbelarien besteht ursprünglich aus individualisirten Zellen. Die Art und Weise der Verschmeltzung dieser Zellen ist eine verschiedene. Bei den Alloicölen und wohl auch einen Theil der Rhabdocoela wenigstens tritt eine Differenzirung in Gerüst und Saftplasma in jeder Zelle ein, und die Zellwandung en resp. die Zellen verschmelzen unter einander. Innerhalb der beiden Gruppen der Dendrocölen und vieleicht bei einigen Rhabdocölen treten zugleich mit der Verschmelzung der Zellen Vacuolenbildungen auf. Diese sind zum

Theil mindestens intercellular bei Tricladen, stets intracellular bei den Polycladen".

Ijima ('84) first recorded the existence of two kinds of cells in the parenchyma. He says (p.385):

"Die so von einander verhältnissig weit entfernten Kerne sind mit anastomosingnden Strängen von Protoplasma umgeben. Mit anderen Worten heist dies die Bindegewebszellen verästeln sich mehr oder minder deutlich und die Aste stehen in Zusammenhang mit einander, wodurch das Reticulum oder die Bindegewebsbalken gebildet werden. Mitunter kommen auch unverästelte Bindegewebszellen vor, welche aber mit reticulumbildenden Zellen durch Zwischenformen verbunden sind". His figures seem to show a structure similar to what I have interpreted in Pl. maculata as a syncitium. Ijima also says that the individual branched cells are more numerous at the periphery of the body than elsewhere, a fact to be compared with my observations, which follow, upon the large number of such cells in Pl. maculata when it is undergoing normal fission.

Woodworth ('91) speaks of the parenchyma of Phagocata gracilis as a "reticulate mesenchym" and does not describe any unbranched type of cells.

Chichkoff ('92) summarizes his views of the paren-

chyma as follows: (p. 490) "Je considere donc le parenchyme des Dendrocoeles d'eau douce comme uniquement compose de cellules a prolongements, qui, s'entrecroisant, se reliant les uns auz autres, determinent de petits espaces lacunaires, ainsi qu'on le sait deja. Mais a côté de ces cellules a prolongements, il n'est pas rare d'en rencontrer d'autres, qui en sont depourvues, revetant une forme plus ou moins ronde, et possedant un noyau avec nucleole bien visible: ce dernier, du rest, se trouve dans toutes les cellules parenchymateuse." Farther on he says that the "cellules sans prolongements" are found in greatest abundance at the peripheral parts of the body. Chichkoff, therefore, separates the cells without branching processes sharply from the cells of the parenchyma proper and says nothing of any intermediate stages.

F. von Wagner ('91) describes in the parenchyma of Rhabdocoeles what he calls "Bildungszellen", which he says are parenchymatous in origin and which are the starting points for all the new organs in regeneration. From his own work and that of others he makes the statement (p. 385) that, "bei den Turbellarien die Regenerationen vom Parenchym (Mesoderm) ihren Ausgang nehmen, das Regenerationsvermäge dieser Thiere somit an die Bildungsfähigkeit dieses

letzteren gebunden erschint". That is, he goes back to the parenchyma as the source of his "bildungszellen" and hence of all the rest and what he calls "bildungszellen" are homologous with the "*univerästalte bindegewebes zellen*" of Ijima, the "*cellules sans prolongements*" of Chichkoff, and the large cells which I have described in the parenchyma of *Pl. maculata*.

That the parenchyma is the tissue from which the new parts come is I think agreed by all who have studied the histological details of regeneration in Planarians (v. Wagner, ('91) Keller, ('94), Van Duyne, ('98); Flexener, ('98) Bardeen, ('01).

Keller, ('94), in his study which is principally upon Rhabdocoeles, although he examined Triclads for comparison, has gone a step further than v. Wagner felt justified in doing and instead of deriving the "Bildungszellen" from the parenchyma he believes that the unbranched parenchyma cells which v. Wagner called "Bildungszellen" and of parenchymatous origin, are almost like a fourth germ layer which remains undifferentiated and from which most of the adult body originally came. He interprets the transitional stages between these "Bildungszellen" or "Stammzellen" as he calls them and the branched cells of the parenchyma retic-

ulumum as the origin of parenchyma from these cells. He explains his theory by a diagram which represents the development of a Planarian after the stage in which the embryo consists of seventy or eighty similar blastmeres as follows: from the blastomeres arise (1) the primary ectoderm, (2) the primary endoderm, forming a kind of blastocoele in which lie a few wandering cells. From these last there arise one after another the secondary ectoderm, the secondary endoderm, the remainder of the parenchyma, the nervous system, gland cells and muscle fibres, the definitive pharanyx, considerably later, the reproductive organs and in case of mutilation the parts which are made good. He further says that from his examination of a number of Planarians he finds that those species having the power of regeneration most marked possess the greatest number of these cells. In view of their supposed primitive character Keller prefers the term "Stammzellen" to von Wagner's "Bildungszellen".

How far our knowledge of the cell lineage of Triclads admits of such a definite interpretation I am not prepared to say. The theory is a suggestive one, though from the nature of the case impossible of actual demonstration. Spindle-shaped cells having the characteristic nuclei and nucleoli of the "stammgellen" do exist in Pl.

maculata embryos at the time when the permanent pharynx is being formed. I should myself be inclined to the more conservative statement which is merely a resumé of the facts, that the cells left between the primary extoderm and endoderm of the embryo produced pretty much everything else and that when the Syncitial parenchyma can be first discovered there are also individual cells scattered through it, which though the larger one may be easily distinguished as such, are connected with the syncitial mass of the parenchyma by every possible intermediate stage, and this series of intermediate stages may be interpreted as "stammzellen" becoming parenchyma or as parenchyma becoming "stammzellen". If the latter be the interpretation these cells would be continually arising from the parenchyma, we should lose the continuity of the "stammzellen" which Keller assumes have come on down unchanged, giving off the others as from an undifferentiated stock and we should then consider the cells of the parenchyma as the final ones to which we can trace the power of regeneration.

The facts which do come out clearly from this review are that we can distinguish (1) the parenchyma proper and (2) certain other individual cells with intervening stages between the two and that the individual cells as such be

their origin early from the embryonic cells or later from the parenchyma are the histological basis for regeneration in the Rhabdocoeles and Triclads which have been carefully studied and that these studies have extended over a sufficient number of forms to show their general occurrence.

In my description of the parenchyma of Pl. maculata I have heretofore referred only to what is found in worms from a locality where there is practically no asexual reproduction. In localities where normal regeneration is going on as a result of the normal amputation occurring in fission the condition of the "bildungzellen" is of particular interest. The number of these "stamm" or "formative" cells, as I shall call them using a translation of Keller's term, is so enormously increased in the dorsal region of the body (Fig. 36 s) above the gut that they are far more numerous than any of the other mesodermal elements. There seem to be more of them throughout the body than are found in worms from localities where fission is not occurring, but this would probably not have attracted attention were it not for the very great number in the dorsal region (Fig. 39 s). These cells both dorsally and ventrally and in all parts of the body in all the specimens during the entire season of normal fission are at all times dividing very

rapidly as shown by the mitotic figures which are everywhere abundant (Figs. 36 and 39 k). It is always the large individual cells which are dividing and never the smaller nuclei of the parenchyma proper. The daughter cells resulting from these divisions have nuclei exactly like those of the parenchyma in size and appearance, but have their own cytoplasm though this is of very small amount. These are the only cells in which mitotic figures have been discovered. Cell division may occur in the endoderm, but I have never seen any indications of it though I have searched through many sections. The same result came from the examination of the ectoderm, though here the rhabdites greatly obscure the nuclei and, as noted above, the small nuclei of the parenchyma proper have never been found dividing. This makes it evident that it is the formative cells which are increasing far more than any others at the time when the worms are making good the parts lost by fission and growing so fast that they frequently double in all their proportions in twelve days (compare Figs. 19 and 32). In worms taken at the same season of the year from localities where sexual reproduction is in progress and fission is practically absent formative cells may be found dividing, but only by considerable searching. What part

these cells play in the regeneration of the new parts after normal fission will appear from the following description of the histological changes found in the head and tail pieces during the four or five days after their separation.

The figures (35, 37, 38, and 41) upon which this description is based are all from sagittal sections of tail pieces which are better adapted to illustrate the facts than sections in any other plane. The changes in the posterior part of the head ends are exactly the same in character minus of course the formation of the organs peculiar to the head and any one of the figures might do as well to represent what occurs in the posterior end of a head piece. In all the figures of Plates 4 and 5, except figures 38 and 39 the anterior end of the animal lies to the left. In figures 38 and 49 the anterior end of the animal lies to the right. Figure 35 represents a sagittal section of the anterior portion of a tail piece such as is shown in Figure 2. Figure 37 is a similar section of a tail piece about thirty hours after fission (Fig. 3). Figure 36 is part of a sagittal section from about half-way between the anterior and posterior ends of a tail piece which has just separated from its head, but it might equally well represent any vertical longitudinal section toward the middle

of any animal from a locality where active fission is occurring. Figure 38 represents a sagittal section through just the tip anterior end of a tail piece on the third day after fission (Fig. 4). Figure 39 is a section similar to Figure 36, but is in the region of the developing pharynx (Fig. 5). In this figure the anterior end is to the right. Figure 40 is a section in which the anterior end lies to the left and shows the pharynx fairly well formed (Fig. 17). Figure 41 is again a section similar to figures 35 and 37 and represents what is found at the anterior tip on the fourth day (Fig. 5).

The conditions found in the naked tip of the tail piece (Fig. 2) a few hours after its separation from the head piece are represented by the sagittal section shown in figure 35. Dorsally, the formative cells (s) are seen to lie in the parenchyma and to be more numerous than elsewhere. They are quite prominent because of their large size and their nucleoli. At the exposed part of the parenchyma there are a considerable number of these cells, but not enough to indicate their special multiplication at this place previous to the fission. The approximation of the dorsal and ventral sides sufficiently accounts for the slight massing of parenchyma and formative elements at this

point. The ectoderm (ec) has been broken and shows rough edges and the parenchyma is left exposed. This may be compared with any of the figures of the outside at this stage (Figs. 2,21 and 25). This section just passes through the inner side of one gut ramus (g). It is at once evident that one could not distinguish such a section from one of a worm which had been artificially cut in two and furthermore that there are now no signs of any histological changes which may have preceded the fission. The formative cells both dorsally and ventrally are actively dividing as is shown by their numerous mitotic figures (k), but the amount of division at the exposed tip is no more extensive than can often be found in a space of the same size anywhere in the dorsal region (Fig. 36). In fact the neighboring sections showed more cell division going on in the dorsal and ventral regions some distance back of the exposed tip, as is shown by the right half of figure 35, than at the tip itself where the new ectoderm is to form.

Flexener ('98), in studying the histological changes occurring in the regeneration after an artificial cut similar to this normal one of fission, says (p. 340), "At the end of the first twelve hours and about equally at the conclusion of eighteen hours active cell proliferation in

the divided end is going on. The evidences for this are found in the rich mitosis encountered as well as in the accumulation of small immature cells at the injured extremity. The most active division is found in the tissues immediately adjacent to the epidermal * * * at the end of twenty-four hours cell division in the regenerating end is quite over. Long and painstaking search is required to discover a single karyokinetic figure".

The artificial cutting seems therefore to have stimulated in Flexener's Pl. maculata (*) a special production of new cells near the cut end during the first twenty-four hours. There is certainly no very well marked period of extremely active cell division after normal fission and this I account for by the fact that an asexual reproducing Pl. maculata always has an excess of the formative cells in all parts of the parenchyma and particularly along the dorsal side and that there is no need for any marked increase of cells at the exposed end nor would there be if the worm divided itself at any other point. There is perhaps a slight increase in the amount of cell division among

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(*) The worm which Flexener calls "Planaria torva" and which is the subject of his paper came from the localities where I have collected my specimens and is incorrectly called "torva" as I have identified it as Pl. maculata.

the formative cells at the exposed tip, but not enough to be readily noticed. It is the same process which Flexener observed only the animal is prepared for its fission by the increased number of formative cells. These cells are always dividing in all parts of the body no matter what stage of regeneration is examined. Their increase would seem sufficient to account for the continual increase of bulk of the whole body and they are present at the normally cut surface ready to make good the loss. I have often wondered if there was not some actual migration of the large formative cells from the more distant portion (see the right side of Fig. 35) toward the exposed end for they seem to lie free in the parenchyma proper and are dividing more actively a short distance behind than just at the cut surface, where in the third and fourth days (Figs. 38 and 41) there is more increase in the number of small nuclei than seems to be accounted for by the division at the tip end itself.

On the second day (Fig. 37) the outermost of the large formative cells have begun to make the new ectoderm (ec') and back of this there is an increasing bulk of syncitial parenchyma with close set small nuclei. As only the large cells divide, and their reconstructing nuclei are exactly like these, this collection of nuclei has presumably

originated from the division and so reduction in size of the large cells. A considerable number of large cells still remain near the tip and mitotic figures are to be found among them. On following the section posteriorly the formative cells are as numerous as ever and their divisions as frequent, though this does not appear in the section drawn (Fig. 37).

There is no change in the old ectoderm (ec) except possibly the loss of some cells at the edge between the new tissue and the old. At the exposed surface the arrangement of the larger formative cells shows at once that they are producing the new ectoderm (ec'). This section cuts through a considerable portion of one of the still unfused gut rami (Fig. 3).

The third day (Fig. 38) shows an increase in the number of small nuclei evidently arising by further division of the large formative cells so that the latter are found in considerable numbers only some distance back. The ectoderm at the exposed tip (ec') is now definitely formed and save for the absence of rhabdites the boundary between the old and the new ectoderm could not be distinguished.The killing fluids often produced clefts between the new ectoderm cells, as shown in the figure, a phenomenon which I

have frequently observed in the delicate ectoderm of embryos killed when about ready to leave the egg-capsule.

On the fourth day (Figs. 5 and 17) a similar section (Fig. 41) through the head shows approximately the mature organization of the parenchyma in this region, there being many small nuclei and the larger cells entirely absent untill a point opposite the gut is reached. This is a section of the anterior end of a tail piece considerably smaller than the others and as it is drawn to the same scale it might at first glance indicate more elongation and flattening of the new tissue than occurs. There is, however, on the third and fourth days a flattening and elongation which does change the shape of the regenerating end as the outlines of the figures show. The commissure (nc) connecting the two halves of the new brain is now seen for the first time.

The formative cells at the exposed tip seem to form as above described the new ectoderm, and the large number of small nuclei of the adult head parenchyma to all appearances come from the division of the nuclei of formative cells the cytoplasm of which becomes continuous with that of the parenchyma syncitium (Fig. 42 sn).

For as much as twenty-four hours after the fission

there is no discoverable change in the parenchyma at the point where the new pharynx is to form. During the second day there appears in this region an irregular massing together of large formative cells. Dorsal and ventral to this mass cells the mitotic figures are abundant, but are not so frequent in the mass itself. The appearance of so many large cells all at once suggests again that some have migrated from the dorsal mass of formative cells. By the third day (Fig. 39) these irregularly directed oval and spindle-shaped cells have become almost all oriented alike and so closely packed that in sections 5 mu in thickness they are sometimes two or three cells deep. This mass of cells (ph') covers the extent of the new pharynx and there cannot be any doubt what cells are responsible for this organ. Between the anterior end of this mass and the gut rami which have now united (Fig. 5) are fewer cells also markedly spindle-shaped and drawn out in the longitudinal axis of the animal (Fig. 39, phl'). Apparently the fibres of the parenchyma about these are also drawn out for they appear differently from those surrounding them and this differentiation marks out the future lumen connecting gut with pharynx. Where it meets the gut (g) there is such an intimate association between the gut and the formative

cells (s) one is forced to the conclusion that these are being added on to the gut. This apparent fusion of the formative cells with parts of the gut will be taken up in detail later. The mass of close packed cells (ph') extends about twice as far back as is shown in figure 39. The cavities of the pharynx lumen and its sheath have not yet appeared. The cavity of the sheath appears a little before the cavity of the mumen being first evident as a split around the periphery of the posterior two-thirds of the mass of formative cells (Fig. 39, ph'), then the lumen begins to appear by a separating of the individual cells in the centre.

Figure 40, in which the anterior end of the animal lies to the left, represents a stage in which the lumen (phl') of the pharynx is forming and not connected with the gut (g) or opening on the free end of the pharynx. The pharyngeal sheath is formed, but the mouth has not broken through. The mass of formative cells of the third day (Fig. 39) is here represented by many rounded cells in the rudiment of the pharynx. Many of these show plainly their cell outline and a nucleus with the characteristic nucleolus of the formative cell. In the centre they have separated for the beginning of the lumen (phl') the epithelial

lining of which is just beginning to form and over this area the parenchymatous matrix has disappeared. The epithelium of the pharyngeal pocket (ep) is already formed, but some of its nuclei still show the characteristic nucleolus of the formative cell. Just anterior to the pharynx in this figure are to be seen what are unquestionably formative cells which are within the gut cytoplasm (g) and in the neighboring sections this approaches almost to the diminutive lumen (phl'). The change from this to the adult pharynx is the completion of the lumen and the breaking through of the mouth. The formative cells which have built the young pharynx lose their identity as such and their nuclei change during the next day or two into the smaller nuclei of the adult pharynx some of them still retaining nucleoli such as are always present in the formative cells and often in the other cells of the body excepting the ectoderm.

The changes in the nervous system have been described by Bardeen (1901), and Flexener ('98). I have nothing to add to their observations.

Regarding the close association between the formative cells and the gut which was mentioned in the stage represented by figure 39, I have these additional observa-

tions. At the anterior end of the gut in tail pieces where the median ramus grows out (Figs. 5 and 17) sections show a very intimate connection between the formative cell and the cells of the gut. The gut (Figs. 35-43, g) in well fed worms has, as is commonly known, the appearance of a syncitium enclosing vacuoles (v) and masses of digesting food. The outlines of the cells are difficult of demonstration. Figure 42 represents a sagittal section through the anterior tip of the gut (see Fig. 16). The anterior is to the right and the magnification Zeiss 1/12 x 2. The parenchyma syncitium (sn) with its own nuclei (np) and also scattered formative cells are shown. Some of the last are removed from the gut, but there are many which are closely applied to it as in the lower half of the figure and even extend up into the gut mass some distance. There are still others which are entirely surrounded by the gut cytoplasm and show in their own cytoplasm small vacules a thing I have never observed in formative cells anywhere else and which seems additional proof that they are being added on to the gut by their cytoplasm fusing with the gut cytoplasm Many of the gut nuclei show a nucleolus exactly like that of the formative cell, but since nucleoli are sometimes found in nuclei of various sorts in the adult and normal

worms one cannot make too much of this. The cases where the formative cells cytoplasm and all are within the gut are the strongest link (see also Fif. 39, g and s). I have observed formative cells seemingly entirely surrounded by gut cytoplasm at other places than the anterior and posterior face of the new median ramus. This is not so remarkable since many small branches are everywhere growing out from the main rami at this time and it would not be strange if the process occurred elsewhere, but I have never seen any such number of formative cells so intimately connected with the gut as at the anterior tip of the new median ramus. As there is no other place where the gut is being remodeled to such a marked degree this is what might be expected.

That the formative cells of the parenchyma become added on to the gut in some places would seem a remarkable thing. The entire absence of observed cases of mitotic figures among gut nuclei during the time when the animal was growing so rapidly and the gut of course likewise increasing would be something at least in favor if not evidence for such a possibility. If it should be objected that neither had mitosis been seen in ectoderm I can call attention to the way in which the rhabdites obscure many

ectodermal nuclei while the nuclei of the gut are readily seen. It is a difficult case to prove, but I can only believe from such instances as are shown in figures 39 and 42 that where the new anterior ramus develops some formative cells are added on and become gut cells. The intimate association of the two seems to admit of no other interpretation and if this is the case one must ascribe a remarkable versatility to the formative cells when their whole history in regeneration is considered.

Whether the stages intermediate between parenchyma and formative cells represent parenchyma originating from formative cells or the reverse I leave an open question. That the formative cells which always occur in Pl. maculata in some number and in very great numbers during the season of fission, are the basis for the regeneration following normal fission there can be no question. The existence of these cells and their role in regeneration in many other Planarians and Rhabdocoeles has been recorded as shown in my review of the subject. The latter has been thoroughly described in Rhabdocoeles, but not figured at all or thoroughly studied in the Triclads. My observations on the existence and role of such cells in Pl. maculata do not therefore stand alone.

Starting with these formative cells which are continually increasing their number (see mitotic divisions in the figures) we get ectoderm, parenchyma, tissue of adult head, according to Flexener ('98) ganglion cells, pharynx, and probably parts of the gut. As far back as we can go, viz:, to the many self-sustaining and multiplying formative cells everything is to be referred to these little differentiated cells which may become almost anything. There is nothing in the process as I have seen it to indicate the degeneration of a highly specialized adult tissue into something simpler and from this the building of new organs. I regret exceedingly that I have not had the opportunity to study and compare what occurs in specimens artificially cut in two at just the place where fission normally occurs. From the description of Flexener ('98) of Pl. maculata and Keller's ('94) statements as to the forms he studied I can hardly see how there can be any different processes carried on in the regeneration after artificial cutting except that the excess of formative cells in animals which are multiplying asexually may make the process easier to follow.

A structure which I have found to a varying extent in almost all the specimens examined from localities where

fission is in progress is shown as x in a number of the figures (Figs. 35, 36 and 41). There are lying in the parenchyma, mostly in the dorsal region, masses of large cells the outlines of which are not always easily discernable. They are not connected with the gut in any way nor do they resemble any structures which I have ever found described for the Planaria. They can best be characterized by saying that they have the appearance of gut cells without any vacuoles, for their nuclei are very much like those of the gut and their cytoplasm stains in the same way, but they are not found connected with the gut in any way. They are furthermore not at all like the first beginnings of the reproductive organs and although I have held many theories as to their significance I cannot offer even a plausible conjecture of their meaning. My reason for mentioning them is that they are usually a striking feature of animals from an asexual locality.

V. THE ANATOMY OF THE FULLY DEVELOPED REPRODUCTIVE ORGANS.

During the summer of 1898, while at the Marine Biological Laboratory, Wood's Holl, Mass., I became interested in studying some very large specimens (15 to 20 mm. in length) of Planaria maculata. These were found in the ponds near Falmouth and with them large numbers of eggs which they lay during May and early June. Reproductive organs were of course fully developed in these specimens and I studied their anatomy casually while endeavoring to ascertain new facts regarding the function of the uterus, penis, etc., during the egg-laying. At this juncture I was surprised to read in a recent paper by Woodworth ('97) the following sentence, "Planaria maculata is the commonest of our fresh water planarians and was the first one to be described (Leidy, 1848, however, nothing has been published regarding its sexual organs, and I am unable to offer anything in this regard; not one of the hundreds of specimens that came under my observation was sexually mature."

Since the anatomy of the most complex system of organs in this common species is entirely unknown or at least unrecorded I have availed myself of the opportunity to

study them in their fully developed state and I shall describe the main features of their structure although there is nothing strikingly different from what has been found in closely related forms. The relative position of the different parts may be seen from Figure 56, though this represents them in an immature condition. The atrial organs are shown by dorsal and lateral views (Figs. 33 and 34). From Figure 56 it is seen that the testes are numerous and extend from the region of the ovaries almost to the tail. Although a few large follicles are to be found extending up between the gut lobes, the rest are packed closely together in the ventral region and never found dorsal to the gut. Their lateral extent is seen in a transverse section of the animal (Fig. 60) to be from a point about half way between the lateral margin and the nerve cord to within a short distance of the median line. In front of the pharynx and behind the genital atrium each nerve cord divides the testes of its side in such a way that in toto preparations there often appear two irregular rows on either side while in the region of the pharynx and genital atrium the inner row of either side is lost. Everywhere they are close together and from the ovaries to the tail hardly a transverse section can be found that does not cut a half dozen or

more. In longitudinal sections they are found closely following one another, there being about seventy-five on either side.

The testicular canals (vasa efferentia) are everywhere found connecting one testis with another or the testes with the vasa deferentia. It has seemed to me in studying these fine canals that there is no reason for doubting that each testis has a definite connection either directly or through intervening testes with the vas deferens of its side. In species with a smaller number of testes it is not difficult to discover the connection of each testis with the vas deferens. In Planaria maculata the testes communicate with one another or with the vas deferens in the same way and it is only their great number which makes the exact connection impossible to establish in many cases.

During the egg-laying season the vasa deferentia (Figs. 33 and 34, v d) may be found as very fine tubes extending from the most anterior testes and everywhere receiving testicular canals. Their diameter increases in the region of the pharynx and swollen with spermatozoa they become the seminal vesicles as in other Planaria. In the live worm they may be seen through the ventral surface as two whitish masses on either side in the region of the

pharynx. Posteriorly, the seminal vesicles narrow suddenly and the smaller tubes thus formed turn with a sharp angle, mount dorsally and then turn ventrally to the base of the penis where each opens into an enlargement (en) of the penis lumen. This lumen is of considerable size and has a blind anterior end, but is drawn out posteriorly into a finer tube and opens by a small aperture on the free end of the penis (p). The penis (Figs. 33 and 34, p) is short and thick, closely invested by the walls of its cavity and pointing downward and backward at an angle of about 45 . Along its median posterior face it joins the atrium by a thin mesentery-like fold so that a sagittal section (Fig. 34) shows as though there were only a shallow furrow around the penis posteriorly. The outline of such a sagittal section is given in Figure 34 and the depth of the atrium immediately on either side of this is indicated by a dotted line. Behind this anterior part which contains the penis the atrium is narrowed abruptly. It is worth noting that the longitudinal axis of the penis does not point in the direction of this opening (Fig. 34), but downward and backward as above described and thus the free end of the penis lies somewhat below the opening through which it must pass when protruded. The contraction of muscle fibres in the

mesenteric-like fold may perhaps aid in guiding the penis out posteriorly. The narrow portion of the atrium soon widens abruptly by sending a larger branch ventrally and slightly backward to communicate with the outside at the genital pore (g p). At the upper posterior angle of the main atrial cavity the vagina (v a) extends dorsally and posteriorly. This short canal which I designate as vagina reaches almost to the basal membrane on the dorsal side, before it splits into the stalk of the uterus running anteriorly and the common oviduct running posteriorly on the mid-line (Figs. 33 and 34). The atrial cavity extends posteriorly a short distance beneath the vagina and is here thrown into one or two large folds which run lengthwise. These folds are glandular and are perhaps homologous with the "musculo-drüsen" organs mentioned by Ijima ('84) in his description of Polycelis tenuis, though they are not constant in shape or occurrence and the cavity of the atrium is no farther complicated than by the division into an anterior portion containing the penis and a posterior which receives the female elements. Besides the secretions of the unicellular glands which surround the atrium and enter the vagina the epithelium lining of the atrium seems to be actively at work producing a secretion which appears as

spherical masses detaching themselves from the cells and covering the surface several layers deep.

The stalk of the uterus as above mentioned extends forward from the vagina along the mid-line as a small tube which in many sections shows indications of contraction for the outline of its lumen is folded. In front of the penis it expands into a spherical uterus such as is found in many Planarians (Figs. 33 and 34 u). During the egg-laying season this cavity when seen in transverse section is as large as the pharangeal pocket and extends right up to the dorsal and ventral basal membranes. Within the uterus I have found what were unquestionably masses of spermatozoa since they had the same appearance as the spermatozoa found in the seminal vesicles. In some instances I also found what appeared to be the egg-capsules in process of formation. Almost every live specimen when seen from the ventral side shows a reddish brown mass in the position of the uterus, as though the uterus contained one of the brown egg-capsules. This color must, however, be due to the color of the uterus itself, for it shows exactly the same when there is no trace of a capsule.

The ovaries (Fig. 56, o) lie about one-fourth the entire length of the animal from its anterior end and are

in the usual ventral position just inside the nerve cord. They are of considerable size often more in diameter than one-half the dorso-ventral thickness of the specimen. Figure 52 represents a transverse section through an ovary and also cuts a part of the oviduct (o). The ova in the sexual season are of all sizes, but the majority of them are very large and toward the centre the largest ones are found entirely free and within the head of the oviduct. Around the periphery are considerable numbers of small nuclei (ctn), whose cytoplasm has apparently gone to form a syncitial framework which extends throughout the ovary and in which the ova are embedded.

There is nothing like a parovarium in the mature worm or at any stage which I have seen of the developing reproductive organs and the ovary is compact without irregular lobes extending into the parenchyma.

The oviducts lead from the outer side of either ovary and extend posteriorly just above the nerve cord. Their walls consist of but a single layer of cells. In some particularly good preparations I am quite sure that I have detected posteriorly directed cilia in the lumen. Back of the atrium the oviducts converge and mount to the dorsal side and as they approach the mid-line turn forward

to unite as the common oviduct the relation of which to the uterus and vagina has been pointed out.

The mature yolk glands are connected with the oviduct everywhere along its length. Only one kind of cell is to be found in the ripe yolk glands and these are loose in the cavity like so many enormous spermatids in a testis. These yolk glands extend everywhere, filling the space on the dorsal side and between the gut lobes so completely that there seems little room left for parenchyma. As they are kept out of the ventral region by the numerous testes the result is that they are dorsally placed. They do not have any definite position like the testes, which remain in that part of the parenchyma where they first appear, but seem rather to have grown in and taken all the space that was left. Anteriorly they extend only a short distance in front of the ovary, but like the testes extend back almost to the tail.

VI. THE DEGENERATION OF THE ADULT REPRODUCTIVE ORGANS.

After the egg-laying, stages in the degeneration of the reproductive organs may be readily found. The Table shown below represents these stages as I have found them. After knowing the development de novo of the organs in small specimens these stages will be seen to represent something quite different from the developmental stages and when the months of the year at which they occur are noted they cannot signify anything but the stages of degeneration. Out of a considerable number of cases I have selected six as showing all that is necessary and these with the month at which they were found are indicated in the first column. In the columns following the presence or absence of the ovaries, yolk-glands, atrial organs, vasa deferentia and testes is indicated and peculiar cases by some comment.

A Table to show the Order of Degeneration of the Reproductive organs in Pl. maculata.

ecimen	date	ovaries	oviducts	yolk-glands	atrial organs	vasa deferentia	Testes	Remarks
1	June	present	present	present	present	present	present	Large specimens just after the egg laying season has passed its height
2	June	present	present	present	present	both traced almost to level of ovaries	absent	Unless a chance mutilation this specimen has undergone fission
3	15th Aug.	present but small	extending back as far as mouth	absent	found only as disconnected parts	absent	absent	
4	Early Sept	each with only about 15 ova. See fig 30 Pl.	extending back to the pharynx	absent	small collection of nuclei	absent	absent	
5	Early sept	very few ova	only on one side	absent	small col: of nuclei	absent	absent	
6	early Sept	3 or 4 ova at position of one ovary	absent	absent	small collection of nuclei	absent	absent	Formative cells collected in small irregular masses along the region above either nerve cord see fig 56

The testes by about the tenth of June, although still large, have a much smaller number of spermatozoa and fewer mother-cells in their walls. They seem to become exhausted by the using up of all the germ cells so that nothing remains but a cleft in the parenchyma which then collapses. Contrary to Chichkoff's ('92) view (p.522) that portions of the testes in the forms he studied remain and produce the new testes of the next year, I cannot find that, in Planaria maculata, any sign of the old testes remains. The disappearance of the testes is therefore the first stage in the degeneration. This may be compared with Woodworth's ('91) mention of what occurs in Phagocata gracilis. He says, p. 30, "while the yolk-glands are still in an early stage of development, spermatogenesis has been completed, the testes have disappeared and the spermatozoa are found filling the vasa deferentia", and again, p. 31, "after the spermatozoa have found their way through the vasa deferentia all traces of the testes disappear". In Planaria maculata the vasa deferentia persist for some time after the disappearance of the testes and are to be found reaching almost to the ovaries as in specimen 2 of the Table.

The third case in the Table is the most important.

Testes, vasa deferentia and yolk-glands have disappeared. The ovaries with their oviducts which extend back as far as the atrium remain intact, but in the region of the atrium are found only the remains of the atrial organs. Figure 61 represents what several consecutive longitudinal sections show in this region. There are some structureless masses at the position of the uterus (u), a cavity (c) at the place where the penis would be normally found and a small pit in the ventral surface (ap) which represents the atrial pore, but there is no connection between these portions. The further degeneration as illustrated by specimens 4, 5, and 6 of the Table shows the reduction of the atrium to a mere collection of nuclei, the disappearance of the oviducts and the reduction of the ovaries to insignificant masses containing only a few ova (Fig. 50). The collection of nuclei at the position of the atrium has the appearance of forty or fifty small parenchyma nuclei such as are shown in figures 42 and 43, np, which surround and are embedded in the edges of a small area that takes a good deal of any plasma stain and is more dense than the cytoplasm of the surrounding parenchyma. The normal fission begins at stages of degeneration when the old ovaries and oviducts and some trace of the atrium still remain

(specimen 3 in the Table), and since the plane of fission is just in the region of the disappearing atrium some cases in which the atrium had entirely disappeared much sooner than in others can be attributed to the early occurrence of the fission.

The Table traces the stages of degeneration down to a condition where very small rudiments of the ovaries remain and sometimes some nuclei to mark the old atrium. There can be no mistaking an old ovary (Fig. 50) for one just developing (Fig. 49), although the number and size of the ova may be about the same in a single section and this condition is hardly questionable as the last stage in the degeneration of the sexual organs. The normal fission, which sets in about this time, by cutting off tail pieces, makes many good sized worms which have no sign of the old organs, and the young of the same year have increased in size to such an extent that they can be no longer distinguished from the worms resulting from the fission of the adults. Specimen 6 shows, however, that the production of the new reproductive organs as fall approaches does to a certain extent overlap with the degeneration of the old. In such cases while portions of the old organs still remain there are cords of cells appearing in the parenchyma above

either nerve cord which later give rise to the testes. Such a cord is shown in Figure 46. The origin and later history of these cords and masses of cells will be taken up under the development of the organs in small worms. Whether the old ovary in original head ends in which it exists ever develops into the new one I cannot tell as the original head pieces are of course like all the other worms externally and none of the specimens section showed anything to give a clue. The majority of the individuals not being original head ends with the remains of an old ovary must of course develop all their organs anew in the fall. This development of the reproductive organs in small worms collected from sexual loca;ities in the early fall is what I described in a preliminary paper last year and will here follow through more in detail.

VII. THE DEVELOPMENT OF THE REPRODUCTIVE ORGANS IN SMALL WORMS.

Lang's ('82) statement that the sex cells of Planarians arise from the epithelium of the gut has so far as I know never been confirmed and I think we may set it down as an error in the interpretation of his data. Beyond this and the mere statements that the testes are at first solid

and that the oviduct begins as a solid rod of cells, he says nothing of the development.

The description of the development given by Ijima ('84) seems to be correct as far as it goes and he indicated the essential parts of the process quite correctly. He says that the development of the atrium and penis is like a repetition of the process by which the pharyngeal pocket and pharynx is formed since it begins as a cleft among a mass of mesoderm cells from the anterior wall of which cleft the penis grows out. Ijima also described the sex cell and the yolk glands as developing in situ from individual cells of the mesoderm.

Woodworth ('91) describes the yolk glands of Phagocata gracilis as originating by outgrowths from the parovarial masses found in that species and disputes Ijima's description of their origin in situ. Chichkoff ('92) inclines to Woodworth's view although with no observations of his own.

Since none of the foregoing work offers a complete description of the development of the sexual organs I submit the description of this process which follows. I must preface this account by saying that I do not know whether the stages I am describing are from egg-embryo worms or

from worms which have been reduced to a small size by a period of asexual multiplication. A reference to the section at the beginning of this paper upon the life history of Planaria maculata in different localities will explain this statement. In the locality numbered (1) p. 9) it will be seen that after the eggs are laid the reproductive organs disappear and the process of fission soon reduces the adults to such proportions that they are indistinguishable from the rapidly growing young. Presumably, the end products of asexual multiplication, being otherwise indistinguishable from the embryos of any year, will develop their reproductive organs in the same way, but until the two have been isolated and reared there is of course no way of certainly ascertaining this. The following description is based upon specimens taken from sexual localities in the fall and I have no way of telling what has been the previous history of any of them. However, the study of some twenty-five or thirty specimens which had been cut into serial sections did not bring to light anything which led me to suspect two courses of development, one for the egg-embryo worms and another for those which are the end products of a season of asexual multiplication.

In September of 1899 I collected small specimens

5-8 mm., in the vicinity of Baltimore which upon examination showed their reproductive organs just developing. These stages have since been supplemented by specimens from the same locality and from the Falmouth ponds in the fall of 1900.

The first indication of the developing reproductive organs is the appearance in the parenchyma along the line just above either nerve cord of rounded or cord-like masses of cells. Formative cells such as have been previously described (Figs. 42 and 43, s) are abundant in this region and their frequent mitotic figures show that they are multiplying rapidly. Such a mass or cord of cells is shown in Figure 46 as it lies surrounded by the parenchyma. At this early stage there can be no question that these masses are made up of formative cells. At a slightly later stage when one can distinguish the incipient testes (Fig. 47) from the cords which are to form the yolk glands (Fig. 44) the cell outlines are no longer visible and although the general shape of the nuclei with the characteristic nucleoli (Figs. 44-47) are the same as those found in the formative cells (Figs. 42 and 43) this alone would not be sufficient to establish the origin of the male and female elements from the formative cells. It is the abundance of

such stages as Figure 6 represents which demonstrates beyond question the origin of the testes and yolk glands from the formative cells of the ventral region.

The stages by which the solid rudiments of the testes (Figs. 47 and 48) produce the mature organs by the division of the cells lying toward the centre (Fig. 48) have been repeatedly mentioned by other workers. When the testis first becomes distinct as such (Fig. 47) the cell outlines cannot be satisfactorily made out except in the dividing or reconstructing cells (Fig. 48) toward the centre. It is the same in the case of the incipient yolk-glands but these, from the time they can be distinguished as yolk-glands, are found with two kinds of nuclei (Fig. 44). In the yolk-gland rudiments it is as though the cytoplasm of the smaller cells were making a stroma in which to swing the large nuclei. A similar condition is noticed in the adult ovary (Fig. 52). At the time the testes are distinguishable as many spheres of cells (Figs. 47 and 48) lying along on either side of the body (Fig. 53) the ovary can be distinguished just in front of the most anterior testis and is quite characteristic in its appearance (Fig. 49). I believe that the ova arise from formative cells though they show their characteristic nuclei so early that their

origin is not as evident as in the case of the testes and yolk glands (Fig. 46). At the stage shown in Figure 49 there are already among the young ova smaller nuclei (ctn) and these I consider homologous with the smaller nuclei found through the connective tissue framework of the adult ovary (Fig. 52 ctn).

The development of the different elements of the reproductive system in relation to one another is shown by the diagrammatic Figures 53-56. Figure 53 represents a stage at which the ovaries (o) and testes (t) can be distinguished. There is as yet no sign of any atrium. At a stage slightly older than the above (Fig. 54) there appears in the region of the genital atrium a cleft in the parenchyma (Figs. 57 and 45). This is preceded by a noticeable collecting of parenchyma nuclei at this spot (Fig. 45, n), but I have not found stages which establish the origin of these from formative cells. Lining the cleft an epithelium is formed. In this stage there is present along the line of the future oviduct a long cord of cells a portion of which is shown in Figure 14. This extends from the region of the ovary backward along above the nerve cord past the beginning of the atrium and well down into the tail. There are at this time no other cell cords of this sort in any

part of the worm except close alongside and connecting with this main stem in places where it is double for a short distance. Such a stage might be represented by a diagram-like Figure 54.

Following this there are to be found along the line of each oviduct (Fig. 55) many cords of cells seeming to have grown out from the original one, at any rate they are connected with it. I can only conjecture how the oviduct is formed as I have not found any interpretable stages. The occurrence of the cord of cells such as is shown in (Figures 44 and 54 along in just the position where the oviduct subsequently appears suggests that this cell cord is in some way transformed into the oviduct. It has occurred to me that such a thing might come about by the migration of the large cells (Fig. 44) out from the main axis leaving the smaller nuclei embedded in their own fused cytoplasm, which subsequently becomes hollowed out to form the oviduct. The masses of larger cells would then remain connected with this as the finger-like processes of the young yolk-glands (Fig. 55) are later connected with the newly formed oviducts. In Planaria simplissima I showed (1900) that the yolk-glands originated as outgrowths from the oviducts and this makes more plausible the origin

of the oviduct in Pl. maculata in the manner I have just suggested.

While I have not succeeded in ascertaining the histological processes by which the oviducts are formed, they are laid down close to if not exactly along the main axes of the cell cords shown in Figure 54 during the time that the young yolk-glands (Fig. 55, y) are appearing. An unquestionable oviduct is found in the stage which Figure 55 represents and posterior to the rudiments of the atrium this newly formed oviduct is continuous with a cord of cells such as in the earlier stage (Fig. 54) extended from the ovary to the tail. The original cords of cells are no longer found in the area occupied by the oviducts. This suggests that the cord of cells shown in Figure 54 has in some way been transformed into the oviduct over most of its length. In Figure 55 the atrium is found far enough developed to have the different parts clearly marked.

In the next figure (56) which is practically adult there remains stretching down into the tail upon either side a branching rod of cells which is destined to become the yolk-glands of the posterior end. It can be readily traced with its branches as indicated in the diagram and becomes continuous with the oviduct where the latter bends

inward to reach the common oviduct. The cavity of the uterus has increased in size, the lumen of the penis opens to the outside and the atrial pore has appeared. This is the adult condition save that the vasa deferentia are not connected with the penis lumen. The yolk-glands are much larger than before and pass from the oviduct up between the gut lobes at frequent intervals. No mention has been made in the above of the condition of the ovaries and testes for they have not changed greatly. Both are larger than at first, the ovaries with more large ova, the testes with their cavities containing spermatids of all sizes. Connecting the vasa deferentia with the penis the adult condition is reached (Figs. 33 and 34).

The stages in the development of the atrial organs can be best shown by a series of diagrams representing them as seen from the side. Figure 57 shows at a the primary cavity in the parenchyma lined with epithelium (Figs. 4 and 5). This cavity takes on the shape shown in Figure 58 in which the uterus (u) is indicated and the position of the penis (p) by an irregular fold. The portion which will later connect with the outside is also seen. The cavity (Fig. 59) assumes more and more the contour of the adult atrium by the further development of the penis in which a

lumen appears and the development of the common oviduct (CO). In later stages the developing oviduct of each side grows out to unite with the common oviduct as shown in Figure 56, and the penis lumen opens on the free end of the penis, but the connection of the vasa deferentia with the penis does not occur until considerably later.

The development of these organs shows that there are two distinct parts (1) the germ cells and (2) the accessory organs about the atrium. The two originate separately and later become connected with one another. There are laid down two cords of cells extending from the ovaries well back into the tail. Anteriorly, in the position of these cords the oviducts appear, while back of the atrium the cords remain the same and become yolk-glands. So far as I can make out the yolk-glands arise as outgrowths of this primary rod of cells. The organs of the atrium begin as a cleft in the parenchyma which gradually becomes differentiated into the several parts. The testes, ovaries and oviducts with the connecting yolk-glands arise in the same place that they occupy in the adult, save that with their increasing size the yolk-glands extend up between the gut lobes and so take possession of the dorsal region. So far as I can see there is no particular area from which the

yolk-glands develop as is the case, according to Woodworth's description, in Phagocata gracilis.

VII. A NOTE ON THE EARLIER EMBRYOLOGY OF PLANARIA MACULATA.

In following through the earlier embryology of Pl. maculata, I have not found that it offers any points of difference from the descriptions of other species given by Ijima ('84) and Hallez ('79), save in one particular. This relates to the development of the pharynx and is explained in Figure 51. This figure represents an embryo which has taken up all the available yolk cells through its embryonic pharynx and larval mouth (ph' and lmo). This yolk (y) is seen within as a mass of disintegrating cells with nuclei. When the taking up of the yolk is just completed the embryo is spherical with its embryonic pharynx at one side, a stage which text-books figures have made familiar to every one. The definite endodermal layer which could at first be distinguished can no longer be made out and the wall of the embryo seems to consist of a greatly flattened ectoderm (ec) and closely applied to the inner side of this scattered mesenchyme cells representing the endoderm and mesoderm. This spherical embryo of Pl. maculata flattens down as is shown in Fig. 51, and the inner cells increase in number along what is destined to become the ventral side of the

adult. Posteriorly a mass (ph') of these cells is especially noticeable and this becomes the adult pharynx by the process which has been often described. It will be seen that the lower flat side of the embryo of Fig. 51 represents the ventral surface of the adult and that the larval mouth (lmo) which leads through the larval pharynx into the gut cavity in on the future dorsal surface. This point has seemed to me worth noting since from the descriptions of Ijima and Hallez the larval pharynx seems to open on that side of the embryo which becomes the ventral side of the adult and to have its longitudinal axis correspond to the vertical axis of the adult. Ijima's Figure 28 of Pl. XXIII is a diagrammatic representation of almost the same stage as my Figure 51 save that his figure shows a mass of ento-mesoderm cells over the area covered in my figure by the embryonic pharynx. As he especially states that all trace of the embryonic pharynx disappears before the adult pharynx begins I think it not unlikely that he may have been mistaken in supposing that the place of the new pharynx was identical with that of the embryonic one, and that the embryonic pharynx of Dendrocoelum lacteum may lie at the point indicated by the mass of cells in Ijima's figure, which is identical with the point at which it is found still per-

sisting in Pl. maculata.

The exact position would be hard to establish in Dendrocoelum lacteum since the first pharynx disappears before the second can be distinguished, but in Pl. maculata where both occur together their relative positions can be at once determined, and since it would be rather strange to have such an entirely different relation in forms so nearly alike in all their general structure as the Planarians I believe the mass of cells indicated at this position in Ijima's figure may also mark the location of the embryonic pharynx in Dendrocoelum lacteum.

Summary.

Planarians collected from several localities and resembling each other so closely that one can hardly believe they are even different varieties of the Planaria maculata, show considerable difference in their life histories in the different localities. In some localities the species seems to have reproduced exclusively, so far as the observations go, by fission, in others, only by the sexual process, while there are still others where both processes occur at different seasons. A possible explanation of this is that the reproduction by fission may replace the sexual reproduction for a term of years though the time (3 years) over which my observations extend is not sufficient to fully establish this.

The characteristic feature of the normal fission occurring in this form is that it takes place without any previous furrow at the place of division or development of organs for the new individuals and hence produces the same appearance as an artificial cut which should separate a normally proportioned specimen at a definite point behind the pharynx.

From a study of the histological details in the regeneration after the normal fission I can find no evidence

that the new parts develop by the transformation of highly organized cells to a simpler type which produce the new organs. My whole evidence would rather indicate that there exist in the parenchyma certain individual cells which can be distinguished from the parenchyma proper and that these are responsible for the new parts in regeneration and also for the sex cells of the reproductive organs.

The structure of the mature reproductive organs of this Planarian is here described for the first time.

The development of the reproductive organs is also described, the facts of which are summarized in the paragraph on page 69.

A note is given on a stage in the embryology of this form to show that the adult pharynx does not originate at the place where the embryonic pharynx degenerates.

Johns Hopkins University, June, 1901.

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DESCRIPTION OF FIGURES.

Plates 1 - 8.

Reference Letters.

a.	atrium
ae p'	epithelium of young atrium.
c.	cavity lined with epithelium at position.of old atrium.
co.	common oviduct.
ct.	connective tissue stroma of ovary.
ctn.	nuclei of this stroma.
ds.	zone of formative cells in the dorsal region.
ec.	ectoderm.
ec'	young ectoderm.
ep.	epithelium.
g.	gut.
gl.	gland cells.
gp.	gland pore.
k.	mitotic figure.
lm.	larval mouth.
m.	mouth.
mf.	muscle fibre.

n.	nuclei.
ne.	nerve.
np.	nuclei of the parenchyma proper.
o.	oviduct.
oy.	cord of cells along line of future oviduct.
p.	penis.
pa.	parenchyma.
ph.	pharynx.
ph'.	young pharynx.
phl'	pharyngeal lumen.
pl.	penis lumen.
ps.	pharyngeal sheath.
r.	rhabdites.
s.	formative cells("stammzellen" of Keller).
t.	testes.
u.	uterus.
v.	vacuole.
va.	vagina.
vd.	vas deferens.
x.	masses of cells found principally in dorsal region.
y.	yolk-glands.
yo.	yolk within embryo.

Plate I.

(The figures on this plate were drawn from the live specimens increased 20 diameters. They have been reduced 3/4 in the reproduction).

Figs. I, 2. A tail piece of Pl. maculata from the dorsal aspect a few hours after the normal fission. Fig. I shows the anterior end drawn together as it is when the animal is at rest. Fig. 2 shows the condition when the piece is moving actively about. The pigment flecks are shown only at the anterior end and also the outline of the gut. All drawn from the living specimen.

Figs. 3-7. - Represent the same specimen as Fig. 2 on the five days following the day of fission. In Fig. 4 the eyes are beginning. In Fig. 5 the gut rami have united anteriorly and the rudiment of the pharynx can be distinguished. In Fig. 6 the development of the new median anterior gut ramus has progressed quite far, but the gut is not shown in the posterior end of the specimen. In Fig. 7 the head is well formed, but the pharynx which is shown without the gut is still too far forward. All from the living specimen.

Fig. 8. - A worm developed from a tail piece like Fig. 2 as it appeared the 8th day after the normal fission. From a living specimen.

Figs. 9, 10. - A tail piece of Pl. maculata the 3rd day after its normal fission and again on the 19th day to show the change of proportions. In Fig. 10, the gut is not shown. From a living specimen.

Fig. 11. - The outline of the posterior end of a head piece of Pl. maculata on the day of normal fission and drawn over this the outlines (2,3,4,6,7) on the six succeeding days. From the living specimen.

Fig. 12. - A tail piece on the day after the normal fission. To show a peculiar condition of the right gut ramus. From the living specimen.

Figs. 13, 14. - A tail piece on the day after fission and again on the third day after. To show remodelling of the gut. From the living specimen.

Fig. 15. - The anterior end of a tail piece on the day after normal fission as it appears when fully expanded.

Figs. 16 - 18. - The same tail piece on the 3rd, 4th and 5th days after normal fission. From the living specimen.

Plate 2.

(All the figures are again from living specimens drawn increased 20 diameters, but are reduced 2/3 in the reproduction).

Fig. 19. - A head piece of Pl. maculata a few hours after normal fission showing the cut expanded as it is when the animal moves about. The gut is shown only in the region of the pharynx.

Figs. 20, 21. - The same specimen as is shown in Figs. 2-7 redivided on the eighth day after the condition shown in Fig. 2.

Fig. 22. - A normally proportioned Pl. maculata just before normal fission.

Fig. 23. - A specimen to show bizarre branching of the gut.

Figs. 24, 25. - Represents the specimen shown in Fig. 22, just after it divided two hours later. In Fig. 24 the gut is shown only in the region of the pharynx.

Fig. 26. - The posterior end of Fig. 19 as it appears contracted when the animal is at rest.

Fig. 27. - The posterior end of a head piece on the day after normal fission.

Fig. 28. - The posterior end of the same specimen as shown in Fig. 19, on the second day after the fission.

Fig. 29. - The same on the fourth day.

Fig. 30. - The same on the tenth day, the gut not being shown.

Fig. 31. - A specimen with bizarre branching of the gut.

Fig. 32. - The outline of the specimen shown in Figs. 19, 28, 29 and 30 which, seventeen days after the condition shown in Fig. 19, has reached normal proportions. The outline of the gut could not be made out. This figure will also illustrate the outline of a normal Pl. maculata.

Plate 3.

(The figures are drawn from a reconstruction of a transverse series of sections and reduced 3/4.)

Fig. 33. - The atrial organs of Pl. maculata leading to them, from the dorsal aspect. The pharynx is shown in outline. The unicellular glands leading into the vagina are indicated by the arrows. The oviduct is drawn without its connecting yolk-glands. Zeiss. A 2.

Fig. 34. - The same from the right side. The oviduct and vas deferens of the left side are omitted. Zeiss. A 2.

Plate 4.

(The figures are from the camera lucida outlines of sections and are reduced 3/4 in the reproduction.)

Fig. 35. - Sagittal section through the anterior portion of a tail piece a few hours after its normal fission

(see Fig. 2 which represents the external features of such a piece). The parenchyma is seen to be naked at the cut surface. The anterior is to the left. Zeiss, D X 2.

Fig. 36. - From the sagittal section of a just divided tail piece (see Fig. 2). The part of the section drawn lies about in the middle of the animal and shows the excess of formative cells with frequent mitotic figures in the dorsal region. The anterior is to the left. Zeiss, D and 2.

Fig. 37. - A sagittal section through the anterior end of a tail piece on the day following fission (see Fig. 3). At the naked end is to be seen what is interpreted as the production of the new ectoderm ec' by formative cells. The anterior is to the left. Zeiss, D X 2.

Fig. 38. - A sagittal section through the tip of new tissue on a tail piece (Fig. 4) of the third day. The new ectoderm is formed and in place of the large formative cells of the first day (Fig. 35) are found many smaller nuclei with no distinct cytoplasmic outline. The anterior is to the right. Zeiss, D X 2.

Plate 5.

(The figures are from camera lucida outlines of sections

and are reduced 3/4 in the reproduction).

Fig. 39. - From a sagittal section of a tail piece on the third day. The part shown is in the region of the newly formed anterior gut ramus and the rudiment of the pharynx. (See Fig. 5). Only the anterior half of the mass of cells (ph') destined to form the pharynx is shown. The anterior end is to the right. Zeiss, D X 2.

Fig. 40. - A similar section through the region of the newly formed pharynx. The sheath (ps) is formed, but the mouth has not broken through (see Fig. 19). The lumen of the pharynx (phl') is just appearing. The anterior is to the left. Zeiss, D X 2.

Fig. 41. - A similar section through the anterior end of a tail piece on the fourth day (Fig. 14). The essential structure of the adult head has been reached, there being now almost no formative cells anterior to the brain (n). The anterior is to the left. Zeiss, D X 2.

Fig. 42. - A sagittal section through the anterior tip of the new median gut ramus of a tail piece (see Fig. 5) to show the close association of the formative cells (s) with the gut (g) at this point. The anterior is to the right. Zeiss, 1/12 x 2.

Plate 6.

(The figures are from camera lucida drawings of sections reduced 5/6 in reproduction).

Fig. 43. - The parenchyma of Pl. maculata showing formative cells (s) and the nuclei of the parenchyma proper (np). Zeiss, 1/12 x 2.

Fig. 44. - Portion of the cord of cells which is found lying along above either nerve cord from the ovaries well down into the tail as in the stage of development represented by Fig. 54. The surrounding parenchyma is not shown. Zeiss, 1/12 x 2.

Fig. 45. - A transverse section through the atrial cavity just after it can be distinguished as such (see Figs. 54 and 57). The surrounding parenchyma is shown. Zeiss, 1/12 x 2.

Fig. 46. - A longitudinal section through one of the short cords of cells which are the first sign of the developing sex cells. The surrounding parenchyma is shown. The cord is evidently composed of formative cells. Zeiss, 1/12 x 2.

Fig. 47. - Section through one of the short cell cords which is now sufficiently advanced to be distinguished as a testis. Zeiss, 1/12 x 2.

Fig. 48. - The same at a slightly later stage. Zeiss, 1/12 x 2.

Plate 7.

(The figures of this plate are from camera lucida drawings of sections and were not reduced in the reproduction).

Fig. 49. - A section through a young ovary just after it is distinguishable as such (see Fig. 53). The ova (o) are shown surrounded by the parenchyma and among them are nuclei (ctn) which are probably homologous with the nuclei of the supporting stroma of later stages. Zeiss, D x 2.

Fig. 50. - A section through an avary of an adult worm which has finished its reproductive season and in which all parts of the reproductive organs have disappeared.except the ovaries and a remnant of the atrium. Zeiss, D x 2.

Fig. 51. - A sagittal section through a larva of Pl. maculata at the stage when the adult pharynx (ph) is just forming. To show the relation of this to the embryonic pharynx (ph'). Zeiss, A x 2.

Fig. 52. - A section through the adult ovary of a Pl. maculata. Showing ripe ova ready to pass into the oviduct the wall of which is cut through (od).

Plate 8.

(The figures of this plate are reduced 2/3 in the reproduction).

Fig. 53. - A semi-diagrammatic figure of an early stage in the development of the reproductive organs. Outlines of body and ovaries and testes from a camera drawing of a toto preparation. The testes are only shown on one side. Zeiss, A^*x 4.

Fig. 54. - Semi-diagrammatic figure constructed in the same way as the last of a stage in which the atrium (a) is just appearing and the cord of cells (oy) has just appeared along the line of the future oviduct. Zeiss, A^*x 4.

Fig. 55. - Semi-diagrammatic figure like the last of a stage at which the oviduct has appeared along the line where the cord of cells previously lay, while with it are connected the incipient yolk-glands (y). The parts of the atrium can be seen and also portions of the vasa deferentia, but the connections of the vasa deferentia and oviducts with the atrium have not been attained. Zeiss, A^*x 4.

Fig. 56. - A semi-diagrammatic figure constructed in the same way as the last of a stage in which practically the adult condition has been attained, save that the vasa deferentia do not connect with the penis. Zeiss, A^*x 4.

Figs. 57-59. - Diagrams to represent the development of the atrial cavity from a simple cleft to a condition which approximates the adult (Fig. 34). The outline of a sagittal section is drawn in and each figure is from an outline with a camera lucida. Zeiss, A x 4.

Fig. 60. - Outline of the organs appearing in a transverse section of a sexually mature Pl. maculata taken midway between the pharynx and ovaries to show the lateral and vertical extent of testes and yolk-glands. Zeiss, A x 1.

Fig. 61. - The outline of a sagittal section of the region of the mouth and atrium of a large specimen in which the atrial organs were degenerating. The structures found in a number of serial sections are all shown. At the position of the uterus some irregular masses (u) further back a tangle of muscle fibres, a small cavity (c) and a pit on the ventral surface (ap) at the position of the atrial pore. These parts are entirely disconnected. Zeiss, A x 2

BIOGRAPHY.

The author of the foregoing dissertation was born at Richmond, Me.. November , 1875, the son of Fanny M. Norton and William C. Curtis. His education was in the public schools of that state until the age of thirteen, and the next three years in the Wasco Independent Academy at the Dalles, Oregon. His final preparation for college was obtained in Tualatin Academy at Forest Grove, Oregon, and he passed the Freshman and Sophomore years of college in Pacific University at Forest Grove. The last two years of his college course were spent at Williams College, Williamstown, Mass., where, in 1897, he received the degree of A.B. with the rank of ϕ.B.K. on the completion of the regular classical scientific course. The following year he served as assistant in Biology at Williamstown and completed the course of study leading to the degree of M.A., which he received from Williams College in 1898. During the past three years he has pursued the course of study leading to the Doctor's degree at the Johns Hopkins University, having been assistant in Minor Biology in 1899-1900 and fellow in Zoology in 1900-1901 and having served for three summers as an instructor at the Marine Biological Laboratory, Woods

Holl, Massachusetts. His published work consists of a paper published in the Johns Hopkins University Circular, June, 1900, entitled "The Anatomy and Development of the Reproductive Organs of Planaria Maculata", a paper published in the Zoologischer Jahrbüchern (1900) and entitled "On the Reproductive System of Planaria Simplissima, a new Species", and the foregoing dissertation. His minor courses at this university have been Physiology and Palaeontology.